

ANNUAL VERSUS BIENNIAL GROWTH HABIT AND
ITS INHERITANCE IN *MELILOTUS ALBA*

HUGH BURNICE SMITH

PART I OF A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE UNIVERSITY OF MICHIGAN

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INTRODUCTION

A most striking fact of general interest presented by a review of the life habits of crop plants and wild plants is the recurrence in family after family of species having the perennial, biennial, and annual habits. So generally are all three habits found in the same family, even though not always in the same species and genus, that one at once sees the impossibility of discriminating between families or even genera on the basis of duration of life. From a genetical viewpoint, it seems that the different life habits must have originated independently in many lines of descent. It is the conclusion of Eames (1911) and of Sinnott and Bailey (1914) that the perennial habit is the oldest. If the annual and biennial habits are derived from the perennial, they must have been developed many times. The various possible changes from perennial to biennial or annual and *vice versa*, and from biennial to annual and *vice versa*, may be simple or complex; but, in view of their apparently high frequency in evolution, they might be expected to be relatively simple. Whether the hereditary basis for growth habit proves simple or complex, an investigation of it is much worth while. It is of importance from an evolutionary standpoint and has, moreover, the special interest that the problem of duration of life always attracts.

The writer is indebted to Dr. A. J. Pieters for furnishing the seed with which the investigation was begun. He is also under great obligation to the Maine Agricultural Experiment Station for offering the facilities for carrying on the greater part of the investigation, and to the staff of the Botanical Garden of the University of Michigan for experimental facilities and assistance.

HISTORY OF ANNUAL *MELILOTUS ALBA*

The history of annual white sweet clover is pertinent to the evolutionary aspect of this study. *Melilotus alba* Desr. var. *annua* Coe is generally thought to have originated by mutation from the biennial form. According to Pieters and Kephart (1921), Professor H. D. Hughes was the first to call particular attention to this variety, but it probably was observed by S. M. Tracy as early as 1898. It is supposed to have originated in Alabama.

¹ Papers from the Department of Botany of the University of Michigan, no. 243.

Thus, although the biennial form of white sweet clover has long been well known, the annual form is apparently of recent origin. It was described and named by Coe (1918).

RELATION BETWEEN HABIT OF GROWTH AND NATIVE HABITAT

The fact that biennial plants, according to Raunkiaer (1918), are most frequent in high latitudes suggests that some of the northern-climate biennials may represent adaptations, selections by natural conditions of favorable genetic changes, on the part of plants which might be either perennial or annual plants if grown in regions having longer seasons. Livingston and Shreve (1921) have shown that the limits of distribution of many herbaceous and palustrine plants lie parallel to the isoclimatic lines for temperature conditions. So an adaptation on the part of plants to live over winter is an entirely natural one. Since perennial plants are supposed to be geologically older than biennials or annuals, a change from perennial to biennial would represent a progressive evolutionary change. Changes of such nature have been suggested by Holm (1925) as having taken place in a considerable number of plants. In this connection the relationship between habit of growth and native habitat of the various species of *Melilotus* is of interest. This relationship is shown in table 1.

TABLE 1. Showing, for Species of *Melilotus*, the Relationship between Habit of Growth and Native Habitat

Species Indigenous to High Latitudes or Altitudes	Species Indigenous to Low Latitudes or Altitudes	
Biennial Habit	Biennial Habit	Annual Habit
<i>M. alba</i> Desr.	<i>M. Kotschyi</i> Schulz	<i>M. alba</i> Desr. <i>annua</i> Coe
<i>M. altissima</i> Thuill.	<i>M. Urbanii</i> Schulz	<i>M. bicolor</i> Bois. & Bal.
<i>M. dentata</i> (W.K.) Pers.		<i>M. arvensis</i> Willd.
<i>M. hirsuta</i> Lipsky		<i>M. elegans</i> Salzm.
<i>M. officinalis</i> (L.) Desr.		<i>M. gracilis</i> DC.
<i>M. polonica</i> (L.) Desr.		<i>M. indica</i> (L.) Lam.
<i>M. taurica</i> (M.B.) Ser.		<i>M. infestans</i> Guss.
<i>M. suaveolens</i> Ledeb.		<i>M. italica</i> (L.) Lam.
<i>M. wolgica</i> Poirlet		<i>M. macrocarpa</i> Coss. & Dur.
		<i>M. messanensis</i> (L.) All.
		<i>M. neopolitana</i> (Brot.) Ser.
		<i>M. speciosa</i> Dur.
		<i>M. sulcata</i> Desf.

This table shows that the biennial species of *Melilotus* are, as a rule, native to regions colder than the regions to which the annual species are indigenous. It is indicated that some species which are biennial in colder regions might become annual in warmer regions. According to Turesson (1922, 1925), habitat types, or ectotypes, are to be considered as products arising through the sorting and controlling effect of the habitat factors upon the genetically heterogeneous species-population.

PHYSIOLOGICAL LIFE HISTORY OF BIENNIALS

The sugar beet affords a typical example of the biennial habit in that it usually grows vegetatively throughout the first season and produces seed the second season. In this plant a dormant period must occur between the vegetative and fruiting stages; else, as shown by Klebs (1903), the sugar beet continues to produce luxuriant vegetative growth and never flowers. There is evidence that other biennial forms are characterized by a necessary dormant period between vegetative growth and fructification. As shown by Gates (1909), certain *Oenothera* biennials grown under tropical climatic conditions in the greenhouse remain in the rosette stage. Hunger (1913) reported the same fact for *Oenothera Lamarckiana* grown in Java. It is known for this class of biennials, which require a dormant period between the two phases of growth, that modification of environmental conditions may bring about behavior as annuals. Harris (1919) states that under certain climatic conditions sugar beets produce seed the first year, particularly if there has been a drought or other condition causing temporary rest in the growth of the plant. Townsend (1909) reports the annual flowering of beets grown in the Mediterranean region. *Oenothera* biennials started early in the greenhouse and grown under temperate climatic conditions produce fruit in one year; thus, low temperature at the start associated with a long enough growing season is, apparently, all that is required to induce development as an annual in this plant. Bailey (1917, 1: 500) states that carrots, cabbage, onions, and celery can be forced to behave as annuals by thick planting. It is believed that the behavior reported by Seifríz (1923) of the flowering habits of certain bamboos which flower after several years of vegetative growth might be explained in the following manner. Such plants may have the first phase of growth indefinitely extended, possibly through failure of the proper climatic stimulation to take place which brings on the final phase. Once the final phase is entered upon, its completion never takes more than a single season.

Not so typically biennial is the habit of growth which characterizes winter cereals. According to Adams (1924), Fröhlich (1919), Fruwirth (1918), Kenoyer (1921), Killer (1919), Takahashi (1924), and Schiemann (1925), no dormant period is necessary between vegetative growth and fructification. Therefore, the so-called winter annuals differ in habit of

growth from typical annuals only in time of maturity. It is considered that their flower buds are physiologically related to buds of annual plants.

The biennial habit of white sweet clover is intermediate between that of winter cereals and that of such typical biennials as sugar beets. In the case of biennial white sweet clover, flowering takes place normally from stems which grow out from crown buds. In this respect, its growth habit is like that of sugar beets; but it is like that of winter cereals in requiring no dormant period between vegetative growth and fructification. Thus a



TEXT FIG. I. Annual *Melilotus alba*.

change from biennial to annual habit in this species may be considered as simply a change in time of maturity. Pieters and Kephart (1921) noted the occasional first-year flowering of biennial white sweet clover grown in Mississippi and Alabama. A similar behavior was noted in tests made by the writer. It was found that when seed is germinated in the fall and the plants are allowed to grow continuously in the greenhouse, they do not send up seed stems from crown buds before flowering, but produce flowers on the part which under usual conditions dies down after the first season of growth. Thus, under conditions for continuous growth, the behavior of biennial white sweet clover is like that of annual plants. Because of this fact, the biennial form of this species is thought to be related in physiological behavior to the annual form. In biennials, according to Terras (1896), the buds in the axils of the cotyledons remain embryonic and develop into crown buds; and in annuals, according to Schulz (1901), the buds in the axils of the cotyledons do not remain embryonic. Biennial *Melilotus alba* is structurally biennial and functionally annual.

It has been shown that in biennial *Melilotus alba* no dormant period is necessary between vegetative growth and fructification. Therefore, in this species time of flowering is a manifestation of duration of life, which in this study is an important subject of interest.



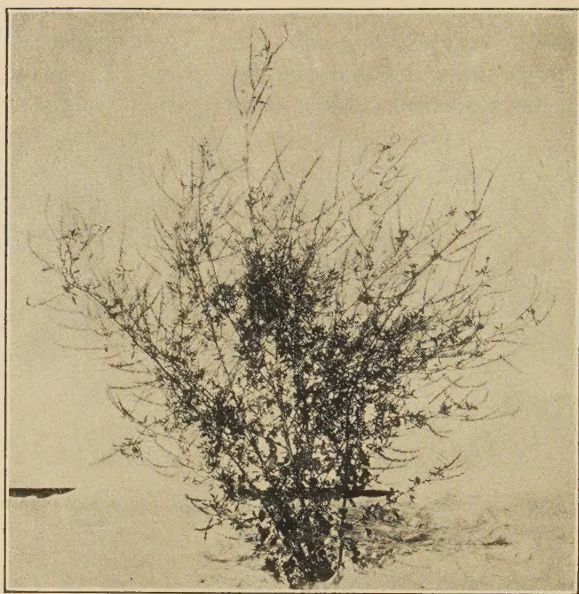
TEXT FIG. 2. Roots of the two varieties of *Melilotus alba*; the biennial form is shown on the left and the annual form on the right.

PHYSIOLOGICAL NATURE OF ANNUAL HABIT OF GROWTH IN MELILOTUS ALBA

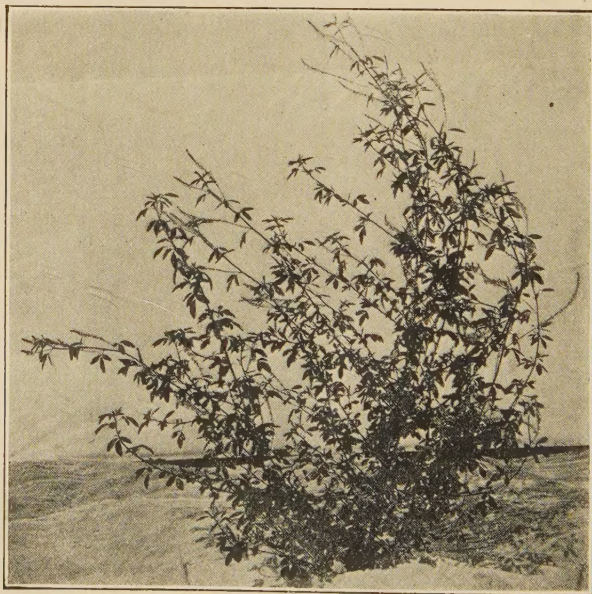
Although certain annual plants sometimes produce more than one generation a year, their life is generally considered as closely connected with the growing season of one year. According to Strasburger, Jost,



TEXT FIG. 3. A biennial *Melilotus alba* plant during its first year of growth.



TEXT FIG. 4. A biennial *Melilotus alba* plant during its second year of growth.



TEXT FIG. 5. An F_1 hybrid between the annual and biennial varieties of *Melilotus alba*.

Schenck, and Karsten (1912, p. 273), the development of fruit in annuals is probably the cause of the death of the vegetative organs. However, annuals sometimes resume growth after fruiting. For example, if annual *Melilotus alba* is grown under conditions for continuous growth, not all the vegetative part dies after the plant fruits; for, if annual plants are transplanted to a cool greenhouse after flowering in the field, they will continue to live after fruiting and will produce seed a second time at least. They live by sending out new growth from latent buds on their flowering stems. However, the greater part of the plant dies after fruiting. A similar behavior in other annual plants was noted by Garner and Allard (1920), who, during their experiments upon the effect of varying the period to which plants are subjected to light, discovered that asters and soybeans could be made to send out new growth after once fruiting. In the ability to resume growth a second time from buds on the flowering stems, the annual form of white sweet clover shows physiological resemblance to the biennial form.

DIFFERENCES BETWEEN FORMS OF *MELILOTUS ALBA*

Melilotus alba is especially favorable material for a study of the physiological nature and inheritance of annual *versus* biennial growth habit because of the pronounced difference between its two growth forms. (See text figs. 1 and 3, representing plants of the same age.) Difference in growth habit, which has been shown to be difference in duration of life, is the principal difference between the two varieties of this species. In comparing other characteristic differences between the two forms, it may be noted that biennial white sweet clover has large roots (text fig. 2), short internodes in the seedling stage, crown buds and succulent texture of stem in the fall of the first year of growth, and fibrous texture of stem in the fall of the second year of growth; and that, in contrast to these characteristics of the biennial form, annual white sweet clover plants have relatively small roots, no crown buds on the roots, and fibrous texture of stem in the fall of the first year of growth. These differences were also noted by Coe (1918), Pieters and Kephart (1921), and Pieters (1925).

Text figure 4 shows the flowering part of a biennial in its second year of growth, and text figure 5 illustrates a hybrid plant from a cross between an annual and a biennial. Hybrids between the two varieties are always annuals. Comparison of text figures 1 and 5 will indicate the degree of difference in luxuriance of different annual plants.

CORRELATION BETWEEN CELL SIZE AND LENGTH OF INTERNODE IN SEEDLINGS

The writer has made measurements of comparable cells in seedlings of the two varieties of *Melilotus alba*. These measurements show that annual plants have larger cells than biennial plants. Pieters (1925) has shown

TABLE 2. Means in mm., Probable Errors, Differences Divided by Probable Errors, and Differences Expressed in Percentage for Comparable Measurements in Annual and Biennial White Sweet Clover

	Annual (A)	Biennial (a)	Difference (A-a)	Diff. P.E. of Diff.	Diff. (%)
Size of stomata of cotyledon.....	0.0402 ± 0.00026	0.0358 ± 0.00026	0.0044 ± 0.00037	11.9	12.2
Size of stomata of first leaf.....	0.0247 ± 0.00016	0.0238 ± 0.00015	0.0009 ± 0.00020	4.5	3.7
Size of stomata of second leaf.....	0.0240 ± 0.00018	0.0224 ± 0.00027	0.0016 ± 0.00032	5.0	7.1
Size of stomata at flowering time of annual.....	0.0232 ± 0.00018	0.0208 ± 0.00018	0.0024 ± 0.00025	9.6	11.5
Cortical cells of root tip, length.....	0.068 ± 0.0012	0.059 ± 0.0011	0.009 ± 0.0016	5.6	15.2
Cortical cells of root tip, width.....	0.057 ± 0.0013	0.047 ± 0.0013	0.010 ± 0.0018	5.5	21.2
Cortical cells of root tip, depth.....	0.042 ± 0.0019	0.035 ± 0.0008	0.007 ± 0.0015	4.6	20.0
Cortical cells of hypocotyl, length.....	0.049 ± 0.0007	0.037 ± 0.0006	0.012 ± 0.0009	13.3	32.4
Cortical cells of hypocotyl, width.....	0.039 ± 0.0010	0.033 ± 0.0008	0.006 ± 0.0013	4.6	18.1
Cortical cells of hypocotyl, depth.....	0.029 ± 0.0006	0.023 ± 0.0005	0.006 ± 0.0008	7.5	26.0
Epidermal cells of root tip, length.....	0.047 ± 0.0009	0.037 ± 0.0012	0.010 ± 0.0013	7.6	27.0
Epidermal cells of root tip, width.....	0.016 ± 0.0003	0.017 ± 0.0003	0.001 ± 0.0004	—	6.2
Epidermal cells of root tip, depth.....	0.014 ± 0.0003	0.013 ± 0.0003	0.001 ± 0.0004	2.5	7.7
Epidermal cells of hypocotyl, length.....	0.049 ± 0.0007	0.037 ± 0.0006	0.012 ± 0.0009	13.3	32.4
Epidermal cells of root tip, length.....	0.060 ± 0.0008	0.057 ± 0.0008	0.003 ± 0.0010	3.0	5.2
Epidermal cells of cotyledon, width.....	0.043 ± 0.0006	0.041 ± 0.0006	0.002 ± 0.0008	2.5	4.8
Epidermal cells of first leaf, length.....	0.065 ± 0.0009	0.056 ± 0.0008	0.009 ± 0.0012	7.5	16.0
Epidermal cells of first leaf, width.....	0.045 ± 0.0006	0.038 ± 0.0007	0.007 ± 0.0009	7.7	18.4
Epidermal cells of second leaf, length.....	0.064 ± 0.0008	0.056 ± 0.0007	0.008 ± 0.0011	7.3	14.3
Epidermal cells of second leaf, width.....	0.045 ± 0.0007	0.038 ± 0.0006	0.007 ± 0.0009	7.7	18.4

that the internodes in seedlings of annual plants are longer than in those of biennials. Therefore, size of cells is positively correlated with length of internode in seedlings. These results are in agreement with those of Brotherton and Bartlett (1918), who showed that length of internode for bean plants grown in the light as compared with length of internode of bean plants grown in the dark is positively correlated with length of primary and secondary epidermal cells. The statistical constants obtained for the various measurements of cell size in *Melilotus alba* are presented in table 2.

Table 2 shows that the size of cells in annual *Melilotus alba* is significantly larger than that of comparable cells in biennial *Melilotus alba* for the following structural parts: size of stomata of cotyledons, first leaf, and second leaf, and leaf at flowering time of annual variety; length, width, and depth of cortical cells of the root tip; length, width, and depth of cortical cells of the hypocotyl; length of epidermal cells of the root tip, hypocotyl, cotyledon, first leaf, and second leaf; and width of epidermal cells of the first leaf and second leaf.

These results and those of Pieters (1925) indicate that, although the chief obvious differences between annual and biennial sweet clover are physiological, nevertheless there are correlated structural differences. Although the differences in cellular organization seem small, it is quite possible that they are sufficient to cause in the plants quite different responses to environmental stimuli. It is to be questioned if sufficiently close investigation would not always disclose morphological distinctions between so-called "physiological" species or varieties.

CHROMOSOME NUMBER OF MELILOTUS ALBA

The two varieties of white sweet clover do not differ in chromosome number; the haploid chromosome number of *Melilotus alba* is 8 and the



TEXT FIG. 6. Haploid chromosome group of *Melilotus alba* at the heterotypic equatorial-plate stage. TEXT FIG. 7. Chromosomes of *Melilotus alba* as observed in a diploid cell.

diploid chromosome number is 16. Although these numbers were first noted by Castetter (1925), the writer, before Castetter published his

results, carried on considerable cytological work and had observed that 8 is the haploid chromosome number of the species. Text figure 6 is a camera lucida drawing of the heterotypic equatorial plate in anther material fixed by Belling's method (1921). Text figure 7 illustrates the chromosomes as observed in a diploid cell. This camera lucida drawing was made from root-tip material fixed in strong Flemming's fluid and stained with iron-alum haematoxylin. The sections were cut $8\ \mu$ in thickness.

INFLUENCE OF ENVIRONMENT ON TIME OF FLOWERING

It is general knowledge that nutrition influences time of flowering; for example, excess of phosphoric acid in the soil hastens time of flowering, and excess of nitrogen delays it. Other factors of the environment which influence time of flowering are temperature and light. Eaton (1924) has shown that soybeans flower much earlier if grown at high than if grown at low temperatures. The influence of light on time of flowering in plants is reviewed by MacDougal (1903), Garner and Allard (1920, 1923), Harvey (1922), Adams (1924), and Tinckler (1925). Tinckler sums up the influence of environmental factors on time of flowering in the hypothesis that for flower-formation to occur the plant must reach a stage of metabolism at which the ratio of carbohydrate to protein lies within certain definite limits. From this it would follow that the factors which influence the time necessary to bring the plant up to a certain stage of metabolism influence duration of life in *Melilotus alba*.

INHERITANCE OF TIME OF FLOWERING

Since in some species, for example white sweet clover, the intrinsic cause for the biennial habit is only time of maturity and is not time of maturity associated with a dormant period, it is desirable to know whether or not time of flowering, especially in those plants which do not exhibit a dormant period, is inherited in a simple or a complex manner. Work to determine this inheritance has been carried out with wheat, barley, oats, rice, peas, cotton, and corn.

Farrer's work (1898) showed that the F_1 hybrids between late and early maturing wheat are intermediate between the two parental types in time of maturity, and that there is wide variation among the F_2 progeny. Biffen (1905) showed further that the characteristic of early ripening is dominant. Thompson (1918) showed that earliness or lateness in wheat is inherited independently of other characteristics and that the earliest F_2 plants are frequently as large and productive as the latest parental type. Freeman (1919), from a study of crosses between durum and common wheats, found that the F_2 progeny of the cross approaches, on the average, more nearly to the late parent than to the early parent. Bryan and Pressley (1921) found that the F_1 plants are intermediate; and that in subsequent generations early maturing biotypes can be isolated by selection. The work of

Aamodt (1922) indicated that the early-flowering habit of spring wheat is dominant over the late-flowering habit of winter wheat. Cooper's work (1923) indicated that in crosses between true spring and winter wheats the short vegetative period is dominant. In a cross between Minnesota no. 169 wheat and three winter wheats he obtained a 3 to 1 ratio in the F_2 ; but if Marquis wheat was used as the spring wheat parent of the cross, a 13 to 3 ratio was obtained. Florell (1924) found monohybrid segregation in the F_2 , and early maturity in spring wheat to be dominant over late maturity.

It is obvious that a genetical change determining time of maturity might bring about either the extinction or the assumption of the biennial habit.

Keeble and Pellew (1910), in their work with peas, showed that lateness of maturity is dominant over earliness. Leake (1911), working with cotton, found the F_1 intermediate between the parental types for time of flowering, and the distribution in the F_2 to be unimodal with the mean nearest that of the late parent. Emerson and East (1913) found that the F_1 of a cross between early pop corn and a late flint corn is intermediate between the two parental types for the time of first exposure of anthers. Hoshino (1915) showed that the F_1 of a rice cross is inclined toward the late parent for time of maturity, and from a study of his material to the fourth generation he was able to explain his results on a three-factor hypothesis. Caporn (1918), working with oats, reported that the F_1 is intermediate between the parents for time of ripening. Tahahashi (1924) and Schiemann (1925), from crosses between spring and winter barley, obtained in the F_2 a 3 to 1 ratio for time of maturity, with the spring habit of flowering dominant over the winter habit. These results indicate that in some cases time of maturity is simple in inheritance, but that in other cases the number of genetic factors involved is not easily determined.

INHERITANCE OF GROWTH FORMS IN *MELILOTUS ALBA*

Methods

The investigation presented in this paper was begun at the Botanical Garden of the University of Michigan in 1922 and was carried on in 1923, 1924, and 1925 at the Aroostook Farm of the Maine Agricultural Experiment Station. Text figure 8 illustrates the experimental plot of 1924 at the Aroostook Farm.

The material for cultures of the annual form was obtained from Dr. A. J. Pieters of the U. S. Department of Agriculture. The seed obtained was representative of the annual white sweet clover as it appears in Alabama, Illinois, Iowa, and Michigan. A representative sample of the biennial form was obtained by transplanting to the experimental plots biennial plants found in the vicinity of Ann Arbor, Michigan.

The general procedure followed was to germinate the seed in the greenhouse about the first of March and to transplant the young seedlings into the field when weather conditions permitted. The plants were grown in cultures representing progenies of self-pollinated individual plants.



TEXT FIG. 8. The experimental plots of 1924 at the Aroostook Farm of the Maine Agricultural Experiment Station.

The technical difficulties, namely, the small flowers and the one-megaspore ovary of *Melilotus alba*, make this species poorly adapted to a strictly genetical study. One beginning work with this species is very much concerned with proper technique for making pollinations, and experiments to determine this technique were conducted by the writer during the summers of 1922 and 1923. A method of emasculating small leguminous flowers with forceps is described by Coffman (1922), but such a tedious method seems unsatisfactory for a long series of experiments. The writer considers the method proposed by Oliver (1910) for making crosses between plants with small flowers to be entirely adequate. Briefly, Oliver's method is that of emasculating the flowers of such plants as sweet clover with a jet of water. Good results may be obtained, by a proper application of this method, with white sweet clover by emasculating the flowers in the afternoon and pollinating during the following forenoon. Pollinations are best made in the forenoon because mature pollen is more abundant in the morning hours than at any other time.

Natural crossing by bees is common in white sweet clover. When biennial plants in their second year of growth are grown near annuals, much crossing results from the visitation of insects, particularly bees.

From a total of 227 pollinations by bees and possibly other agencies, 126 resulted in hybrid plants. In other words, the chances for intervarietal pollination of sweet clover by bees are about equal to the chances for intravarietal pollination. Therefore, to insure intravarietal pollination it is necessary to cover the flowers with some material which will keep bees away from the flowers. Throughout this investigation, the flowers to be selfed were protected by means of no. 2 manila paper bags.

Certainty of self-pollination of hybrid plants was very essential to this study. In order that sweet clover flowers be self-pollinated, the staminal column must be released from its enclosure within the keel of the flower. This operation, called "tripping," is often accomplished in nature by bees. But, since bees must be kept away from flowers to be self-pollinated, the flowers must be tripped by other means. In this investigation the flowers were tripped by rolling them gently with the hand. Since sweet clover is indeterminate in growth, the procedure of removing the bags, tripping the flowers, and replacing the bags must be repeated about every week for four or five weeks in order that sufficient seed be obtained. Great care must be taken to be sure that there are no holes in the bags, otherwise bees are likely to cause contamination.

PRESENTATION AND ANALYSIS OF RESULTS

For determining the inheritance of the annual *versus* the biennial character of white sweet clover, all individuals in cultures of self-pollinated heterozygous plants were noted. The data for this inheritance study are presented in table 3, in which they are listed according to culture number. Annuals are recorded in column A, and biennials in column a.

A total of 2,042 individuals in cultures segregating for the annual *versus* the biennial character have been observed; of these, 1,563 were annuals and 479 were biennials. The deviation from the theoretical 3 to 1 ratio is only 1.7 times the probable error. The significant 3 to 1 ratio obtained means that the difference in habits of growth between annual and biennial white sweet clover is due to a single gene difference in the germ plasms of the two varieties. Consequently, one gene determines the difference in duration of life between the two varieties of *Melilotus alba*. This gene is for time of maturity and not for a dormant period between the vegetative and the fruiting stages. It might also, and quite logically, be considered as a gene for larger cell size in the annual variety, since there is a correlation between the physiological and morphological characteristics of the plants. Why a plant made up of larger cell units should flower after a shorter number of cell divisions is unknown, but this is just what is found in the case of the *gigas* mutations in *Oenothera*, where larger cell size is associated with a smaller number of cell divisions before maturity is attained (Tupper and Bartlett, 1916). According to the hypothesis advanced by Tinckler (1925), previously referred to, time of flowering is

determined by metabolic rates. A single gene, therefore, determines the difference in time necessary to bring the plants of the two varieties up to a hypothetical stage of metabolism at which the ratio of carbohydrate to protein lies within certain definite limits. This ratio may be the same or it may be different for the two growth forms of *Melilotus alba*. This hypothesis is referred to again because it suggests an attractive subject for further study, namely, a biochemical comparison of the annual and biennial varieties, under identical conditions, at various ages. It would be quite in line with expectation to find size of cells, rate of metabolism, and time of maturity all interrelated.

TABLE 3. Ratios Listed According to Culture Number, Annuals Recorded in Column A, and Biennials in Column a

Culture	A	a	Culture	A	a	Culture	A	a
4-3....	18	6	4-133...	18	4	5-75....	18	7
4-11....	33	7	4-138...	12	2	5-76....	19	5
4-14....	38	12	4-139...	16	5	5-79....	47	8
4-15....	19	5	4-143...	11	2	5-80....	8	7
4-16....	39	7	4-144...	15	3	5-81....	18	3
4-21....	30	6	5-1....	13	2	5-82....	8	8
4-22....	22	17	5-16....	17	5	5-83....	3	6
4-25....	33	6	5-17....	10	5	5-87....	8	2
4-29....	30	8	5-24....	6	2	5-88....	13	6
4-30....	37	4	5-25....	17	1	5-89....	10	8
4-32....	31	13	5-26....	11	5	5-90....	7	4
4-34....	22	3	5-27....	11	2	5-91....	8	2
4-37....	33	1	5-28....	7	2	5-92....	25	10
4-39....	20	5	5-29....	6	1	5-93....	10	1
4-20....	27	5	5-30....	10	2	5-94....	10	12
4-21....	28	3	5-31....	16	3	5-101...	10	2
4-43....	5	1	5-32....	7	9	5-102...	7	3
4-45....	28	1	5-33....	13	3	5-103...	10	2
4-47....	31	14	5-34....	4	4	5-105...	7	4
4-48....	53	10	5-35....	5	4	5-106...	10	3
4-50....	30	6	5-37....	41	2	5-107...	15	18
4-86....	10	1	5-38....	14	2	5-108...	9	4
4-90....	32	2	5-41....	6	4	5-109...	26	6
4-98....	18	4	5-63....	31	8	5-110...	18	8
4-103...	18	5	5-64....	5	3	5-111...	5	3
4-104...	21	3	5-65....	19	9			
4-107...	14	3	5-66....	4	3	Total...	1,563	479
4-108...	21	4	5-69....	10	7			
4-113...	30	23	5-70....	7	3			
4-124...	5	5	5-71....	6	7			
4-126...	17	2	5-72....	10	5			
4-128...	20	4	5-73....	10	8			
4-130...	21	2	5-74....	12	12			

DISCUSSION

The question of the origin of the annual form of white sweet clover by mutation from the biennial form has been discussed by Pieters and Kephart (1921) and by Castetter (1925). Evidence presented in the present paper indicates that the difference between the annual habit and the biennial

habit of growth is due to a single gene difference in the germ plasms of the two varieties. The direct origin by mutation of one form from another is more likely if there is a single gene difference between the two forms than if there is more than a single gene difference between them. Because of the close relationship between length of season and habit of growth, a genetic change, a mutation, determining time of maturity may have brought about the assumption of the annual habit. The evidence presented in this paper of a single gene difference in the germ plasms of annual and biennial forms of *Melilotus alba*, together with the recorded history that the annual is of more recent origin than the biennial, seems to indicate that the annual originated by mutation from the biennial. If so, it is a case of direct evolutionary change which has taken place under natural conditions. By a similar simple mutation, the origin of annual from biennial and perennial habit and of biennial from perennial habit in many lines of descent might be explained. Not to generalize beyond the genus *Melilotus*, we should have an explanation of the high correlation of growth habit with length of growing season shown in table 1, summarizing the geographic distribution of the species in nature.

SUMMARY

As determined from a classification of 2042 individuals, all of which were progeny of self-pollinated heterozygous plants, the difference between annual and biennial habits of growth in white sweet clover is due to a single gene difference in the germ plasms of the two varieties. Therefore, a single gene determines the difference in the duration of life of the two varieties of *Melilotus alba*. This gene determines time of maturity and is not concerned with the dormant period between vegetative growth and fructification.

Because of the close relationship between length of growing season and habit of growth in *Melilotus alba*, a genetic change, a mutation, determining time of maturity may have brought about the assumption of the annual habit in this species. It is not known whether the annual white sweet clover originated by mutation from the biennial form; but if it did, it is considered to represent a progressive evolutionary change which has taken place under natural conditions. The evidence presented in this paper of a single gene difference between the germ plasms of annual and biennial *Melilotus alba*, together with the recorded history that the annual is of more recent origin than the biennial, seems to favor the view that the annual form of this species has originated by mutation from the biennial form.

If, as seems to be the case, the origin of the annual was by mutation, we have an interesting addition to the list of dominant mutations.

The origin of annual from biennial and perennial habit and of biennial or annual from perennial habit in many lines of descent is probably to be

explained by mutation. These changes, in view of their apparently high frequency in evolution, may be expected to show simple inheritance. There is a high correlation between the habit of growth and the length of the growing season of the species of *Melilotus* in nature, which conforms to the ideas of Türesson regarding the natural selection of ectotypes.

Biennial white sweet clover is functionally an annual, in that it is capable of flowering on the primary stems of the first season if the period of growth is sufficiently prolonged. It is likewise from a structural standpoint a typical biennial in that it has morphological provision, in its crown buds, for the completion of its life cycle in the second season if (as is always the case in the North) the stems of the first season do not reach maturity.

Annual white sweet clover differs from the biennial most obviously in the shorter period required for maturation. This physiological peculiarity is found to be correlated with a larger cell size in corresponding tissues, which may in turn, if Tinckler's ideas are correct, be associated with a difference in metabolism. (The biennial and annual forms would provide ideal material for a test of the metabolism hypothesis.)

Since the biennial habit of *Melilotus alba* is facultative, it follows that the present paper is primarily a study in the inheritance of duration of life. That a normal difference in duration of life of such great magnitude should be inherited as a simple Mendelian character is a most striking fact.

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